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Interactions with the Soil Fauna

A. H. Fitter and I. R. Sanders

SUMMARY. **Soil animals are known to have important effects on the population dynamics of soil fungi, but their interaction with mycorrhizal fungi has not been extensively studied. Mycorrhizal fungi may comprise a large proportion of the soil fungal biomass, however, and it is to be expected that the animal–mycorrhizal interaction is of considerable significance. The spatial and temporal patterns exhibited by the fungi and soil animals mean that they come into direct contact frequently, and there are observations of grazing by both collembola and aphids. Grazing of external mycelium by soil animals might limit its development, simply disconnect it from the internal or attached mycelium, or even stimulate fungal growth. In the latter case, there might be benefits to the host plant, but in either of the other cases, the consequences for the host are likely to be deleterious. There is experimental evidence that such interactions do occur, and grazing has been shown to eliminate any benefits derived by the host plant from the symbiosis. Very little work has yet been performed under field conditions, however, and the ecological significance of mycorrhiza–fauna interactions remains uncertain.**

Introduction

The soil is a region of great biological complexity and often also of intense activity. In particular, trophic interactions in soil appear to be more complex than in many aboveground habitats, with food-webs apparently having greater connectivity. Coleman (1985) displays a food-web model in which chains with up to 8 links appear possible, for example, roots–bacteria–flagellates–amoebae–omnivorous nematodes–predatory nematodes–nematophagous mites–predatory mites. Since the latter are presumably prey for larger organisms, it is possible to envisage as many as 10 links in a chain. It is unlikely that significant energy and material flows occur through such long chains, but their possible existence indicates that the structure and functioning of belowground communities may be controlled in importantly different ways than those above ground.

The principal source of energy to belowground communities is normally roots, and a significant proportion of the carbon sent to the root system by many plants is diverted to mycorrhizal fungi. These fungi therefore represent an important link in the belowground food-web that has hitherto received little attention. The soil fauna contains numerous fungivorous species, particularly in the nematodes, mites, and collembola, that are potentially important consumers of mycorrhizal fungi.

Nematodes are small (mostly in the range 0.1–1.0 mm), unsegmented worms that feed either by stylets inserted into the food or by ingesting it through an enlarged mouth. The latter is commonest among carnivorous species. Because of their size range, they can be important feeders on plant cells and on fungal hyphae, both of which tend to have diameters around 0.01 mm. Collembola are minute, wingless insects ranging from 0.1 up to nearly 10 mm in length; mites (Acarina) are arachnids with a similar size range, of which the most important fungivorous group is the Cryptostigmata. Both cryptostigmatid mites and collembola have biting mouthparts.

In this chapter we assess the evidence that these soil animals interact with mycorrhizal fungi sufficiently strongly to affect the interaction between fungus and host plant. Because most work on mycorrhizas has been performed in sterilized soil under laboratory conditions, where necessarily animals are excluded, few workers have been aware of the potential significance of the interaction. Since so little work has been performed on interactions between the soil fauna and mycorrhizal fungi, we shall examine first the effects of animals on soil fungi in general, seeking general patterns of response.

Population Patterns of Mycorrhizas and Animals

Predation and Dynamics in Nonmycorrhizal Fungi

The interactions between the major components of the soil—roots, soil fauna, and microorganisms—are complex (Coleman et al., 1978; Coleman, 1985), and the interaction between soil fauna and fungi must be considered in the context of the complete ecosystem. Hunt et al. (1987) have used a systems approach to this problem, identifying the belowground food web in a short-grass prairie. Other workers have concentrated in greater detail on the importance of specific interactions on the cycling of phosphorus (Stuart & McKercher, 1982), nitrogen (Parton et al., 1984), and carbon (Elliot et al., 1984) and also the consequences of these interactions on population dynamics (Anderson, 1978; Moore, 1988; Seastedt et al., 1988).

Predation on soil fungi by soil fauna can directly influence the dynamics of both of these components while indirectly influencing other components of the soil biota and processes within the soil, e.g., nutrient cycling (Anderson & Ineson, 1984). The mechanisms by which soil fauna affect the composition of fungal communities have been classified by Visser (1985). These are comminution, or the fragmentation and mastication of plant debris which includes microflora growing within plant residues, grazing of the microflora, and dispersal of microbial propagules. These direct interactions bring about changes to the composition of the soil microflora and fauna with respect to the size of populations present, the relative abundance of species, and their spatial distribution.

Direct grazing of fungi could either be deleterious by reducing fungal biomass or advantageous by the stimulation of greater productivity. Both of these situations could, in turn, lead to changes in respective grazer populations. The latter, termed direct nonsymbiotic mutualism (Moore, 1988), is thought to be caused by the removal of growth-inhibitory, secondary metabolites produced during hyphal senescence. This enhanced fungal growth and activity has been observed in arthropod–fungus (Hanlon, 1981) and nematode–fungus interactions. Whether grazing becomes mutualistic depends on grazing pressure, growth rate and nutritional quality of the grazed organism, and the success of its defence strategy (Visser, 1985).

Feeding preferences of soil fauna could also be significant in determining fungal species composition. Selectivity of some grazers to different fungal species could result in competitive advantages arising for less preferred species. The collembolan *Onychiurus subtenuis* appears to be important in the competitive interaction of two commonly occurring litter fungi, Basidiomycete 290 and Sterile dark 298 (Parkinson et al., 1979). In experimental microcosms Basidiomycete 290 was more successful in colonising leaf macerate than Sterile dark 298, in the absence of *O. subtenuis*. However, in the presence of *O. subtenuis* the colonising ability of Sterile dark 298 was significantly reduced; in other words, *O. subtenuis* reinforces an existing competitive relationship.

Grazing activity could also influence the spatial distribution of fungal populations. Newell (1984a) has shown that in the presence of two coexisting decomposer basidiomycetes *Mycena galopus* and *Marasmius androsaceus*, the collembola *Onychiurus latus* shows a grazing preference for the latter. In laboratory experiments, *M. androsaceus* was able to colonise litter from the L and F1 horizons more than twice as fast as *M. galopus*, although in the field *M. androsaceus* was restricted almost exclusively to the L horizon. Further experiments investigating the interactions of these three species in the field (Newell, 1984b) support the hypothesis that selective grazing by collembola alters competition between these two fungal species. This may be an important factor in determining their vertical distribution within leaf litter.

Fungal Biomass

There is clear evidence for the interaction between soil animals and fungi, but the evidence reviewed above refers exclusively to saprotrophic fungi. Such fungi are likely to be an important food source for many fungivorous soil animals; whether mycorrhizal fungi are a significant resource will depend in part on their contribution to the total soil fungal biomass. This has been estimated by direct measurement of fungal biomass occurring in the soil, and by calculations based on other variables such as whole plant and root biomass production and the allocation of carbon to roots.

Direct measurements of vesicular-arbuscular mycorrhizal (VAM) fungal spore biomass have been recorded from pot experiments. Sieverding et al. (1989) have extrapolated values recorded in pot experiments to field conditions. They estimate dry matter production in tropical soils to be 20–200 kg/ha/yr, although extreme values could be as high as 1000 kg/ha/yr. The uncertainty associated with such extrapolations is large. Dry weights of VAM fungal hyphae have also been measured in pots for different fungal species (Sanders et al., 1977; Bethlenfalvay et al., 1982). However, obtaining an accurate measure of VAM fungal biomass in natural ecosystems is difficult, since this involves measurements or estimations of both the internal and extramatrical hyphae. The proportion of the internal and external components of mycorrhizas will also be important in relation to the cost to the host plant in terms of carbon allocation.

It has been suggested that the total fungal biomass of VAM is between 5 and 20% of root weight (Smith & Gianinazzi-Pearson, 1988). If this figure is considered together with values for productivity and allocation of resources to plant roots then such a value would suggest that VAM comprise a large resource within the soil ecosystem. For example, in woodland ecosystems root biomass comprises 15–25% of total tree biomass, while the percentage biomass of roots in grassland and tundra ecosystems attains 75–98% of total biomass (Fogel, 1985). Other studies have considered the cost to the plant in terms of carbon allocation to mycorrhizas. It has been estimated that between 6 and 10% of total plant photosynthate is diverted to mycorrhizas (Snellgrove et al., 1982; Koch & Johnson, 1984). However, the total amount of carbon that is diverted cannot be considered as being an available resource. Roots that contain mycorrhizal fungi have a higher respiration rate than uninfected roots. Part of this increase can be attributed to the fungi and part is suggested to be a response in infected plant tissue (Smith & Gianinazzi-Pearson, 1988). Thus, it is likely that although figures for carbon allocation to mycorrhizas are high, only part of this large allocation of resources to mycorrhizal fungi is available to mycorrhizal grazer organisms.

The biomass of ectomycorrhizal (ECM) fungi has also been investigated in relation to the productivity of coniferous forests (Fogel & Hunt, 1979, 1983). In stands of Douglas fir *Pseudotsuga menziesii*, ECM have been shown to comprise 6% of total tree biomass and the mycorrhizal standing crop is estimated to range from 1 to 25 metric tons per ha (Fogel, 1985).

It appears that both VAM and ECM constitute a large, available resource for potential grazer organisms in the soil ecosystem. In grassland and forest ecosystems a substantial proportion of the net primary productivity is diverted to mycorrhizas. However, further investigation is required into how much of this carbon allocation results in fungal biomass, and how much of this is a suitable food source for fauna that may graze on it.

Palatability of Mycorrhizal Fungi

Although it is clear that an extensive amount of mycorrhizal fungal biomass occurs in the soil, it is not known how much of this resource is suitable for or palatable to consumers. The only study investigating the palatability of mycorrhizal fungi has been undertaken by Shaw (1985, 1988). Shaw (1988) carried out a series of choice experiments among pairs of 12 different fungal species (8 of which were ectomycorrhizal) using the collembola *Onychiurus armatus* as the grazer organism. By subtracting the number of fungal isolates that were significantly less preferred from those that were significantly more preferred, Shaw (1988) was able to establish the relative palatability of the 12 fungal species to the collembola. These were then ranked to give palatability rankings or a hierarchy of preference. Of the 12 fungal isolates, the ectomycorrhizal ones ranged from being the most palatable, i.e., *Lactarius rufus*, to those that are relatively unpalatable, i.e., *Pisolithus tinctorius* and *Hebeloma crustuliniforme*.

This investigation gives no indication of what factors affect palatability, although Shaw (1988) suggests that less preferred isolates may allocate a larger proportion of their metabolites to herbivore deterrence. However, the results of such choice experiments must be considered with caution. It is possible that a species of ECM-forming fungus could differ in its palatability when grown in culture and when growing in symbiotic association with plant roots. Additionally, in Shaw's choice experiment, *O. armatus* was only given the choice of ectomycorrhizal fungi and other fungal species occurring in that particular soil. Thus, although the results show that ECM are comparatively palatable to collembola, with respect to other fungi in the soil, it does not provide insight into whether this group of soil-dwelling animals would readily consume this resource in the natural environment.

Although the palatability of VAM fungi has not been investigated, hyphae of these fungi have been recorded in the guts of many animals suggesting that they are a worthwhile food source. However, this assumes that ingested hyphae are subsequently digested: although many soil animals also ingest VAM fungal spores, some of these pass through the gut and have been shown to be viable after extraction from the animal's faeces (Rabatin & Stinner, 1988).

Spatial and Temporal Patterns of Mycorrhizas and Animals

Since mycorrhizal fungi are a readily available and apparently palatable resource, they are likely to be grazed. The extent of grazing would depend on the spatial and temporal patterns of roots, mycorrhizas, and the relevant soil fauna in soil.

Traditionally, studies of the patterns of these organisms have been either from a zoological or botanical/mycological viewpoint; only a few more recent studies have attempted to consider the patterns of these organisms jointly. Much of the literature concentrates on soil animals and VAM and few studies have investigated the spatial and temporal patterns of soil fauna with ECM.

The spatial and temporal distribution of cryptostigmatid mites has been investigated in detail. Usher (1976) showed that these mites form distinct aggregations and that their spatial distribution was nonrandom. A recent study by Fogel and Lussenhop (unpublished data) investigated the distribution of soil invertebrates in relation to plant roots within the soil. They took a census of soil invertebrates along transects at three different times of the year. The observations were made through the windows of a soil biotron (a large root observation chamber) and record the density of individuals occurring in the soil and on plant roots in a mixed northern forest in Michigan. They found that the density of Collembola, Acarina (Tydeidae), and Enchytraeidae was consistently higher on roots than in the bulk soil during July, August, and October, to the extent that these animals were almost exclusively associated with roots. Other invertebrate groups such as the Nematoda, Acarina (Mesostigmata and Oribatida), and Protura also exhibited higher densities on roots than in the soil, although these were not consistent throughout the period of study.

Studies of spatial and temporal patterns of VAM have shown that they are abundant in a variety of natural ecosystems although levels of infection vary greatly throughout the year (Brundrett & Kendrick, 1988; McGonigle, 1987; Rosendahl et al., 1989) and between different plant species. However, at present, patterns of VAM occurrence are not well understood since none of the published data regarding these patterns covers more than 1 year.

A thorough investigation of both temporal patterns of VAM, roots, and soil microarthropods has been carried out by McGonigle (1987). He observed changes in coarse and fine endophyte infection in the roots of eight species of a seminatural grassland in North Yorkshire, England, for a period of 1 year. At the same field site, he also recorded the spatial arrangement of endophytes on the plant roots and the density of collembola and acari at different depths in the soil. Mycorrhizal infection was largely restricted to the top 6 cm of the soil, and the low density of roots below this level implied very low populations of VAM fungi. Collembola and acari also occurred at their greatest densities in the top 6 cm of the soil, supporting the supposition that the grazing of VAM is ecologically realistic.

However, McGonigle's survey shows only that the interaction can occur, since no gut content analyses were carried out to show that these animals were eating fungal hyphae from the soil. It is possible that the collembola and acari were not utilizing VAM fungi and that the aggregation of these animals in the top 6 cm of the soil occurred because they were exploiting another available resource, e.g., plant roots, other soil animals, or other organisms associated with the rhizosphere. The omnivorous nature of some soil fauna that have been considered as fungivorous has been demonstrated. For example, Walter et al. (1986) have shown that fungivorous mites from a short–mid-grass prairie ecosystem will readily consume nematodes.

Other evidence exists for the co-occurrence of VAM and possible grazing animals. Rich and Schenk (1981) have shown that seasonal changes in VAM fungal spore density in the soil are similar to changes in the density of plant-parasitic nematodes. However, VAM fungal spore density is not a reliable measure of VAM occurrence. The association of endoparasitic nematodes and VAM fungi appears to be complex. Evidence shows that these organisms do occur in close proximity and can have significant effects on population densities and VAM sporulation. However, they appear to be mutually inhibitory; mycorrhizal fungi do not colonise roots infected with nematodes or *vice versa* (Ingham, 1988).

Evidence for the co-occurrence of likely mycorrhizal grazers and ECM fungi is extremely scarce. Cromack et al. (1988) found that arthropods and nematodes were more abundant on rather than off ECM mycelial mats (*Hysterangium setchellii*), though with no apparent difference in biomass (this is probably a sampling problem rather than a real difference in response between numbers and biomass), and both Riffle (1967) and Sutherland and Fortin (1968) demonstrated nematode feeding in culture. There are several other, rather anecdotal, accounts of animals feeding on ECM fungi, but as yet no systematic studies have been attempted. To get a clearer understanding of the interactions between soil fauna and ECM more detailed investigations are required into the spatial and temporal patterns of these organisms and the possible consequences of their interactions.

Direct Evidence of Grazing on Mycorrhizal Fungi

Although it would seem that finding direct evidence of grazing on mycorrhizal fungi should be relatively easy, surprisingly few studies have been able to demonstrate this, perhaps because such observational science is no longer fashionable. A number of investigations have shown that the ingestion of VAM fungal spores occurs in a variety of different animals (e.g., Rabatin & Stinner, 1985, 1988), since they can easily be identified from gut contents or faeces. In most cases spore digestibility or palatability has not been investigated. To distinguish VAM fungal hyphal remains from other fungal hyphae in gut contents is more difficult. For example, the results of Warnock et al. (1982) imply that grazing of hyphae occurred. In this investigation greater numbers of the collembola *Folsomia candida* from +VAM pots were shown to contain fungal hyphae than those from −VAM pots. Although it appears likely that the hyphae were of mycorrhizal origin this could not be proved. With the development of techniques whereby mycorrhizal fungi can be identified by isoenzyme separation, following electrophoresis, the future identification of VAM hyphae in gut contents may be possible.

Consequently, evidence for hyphal grazing has relied on direct observation or experiments that have unavoidably created a highly artificial experimental environment. Of these, the observation of aphids (probably *Pemphigus piceae*) feeding on ECM of *Pseudotsuga menziesii* (Douglas fir) (Zak, 1965) is one of the only records of direct grazing on ECM. The investigation by Shaw (1988) also confirms that grazing of ECM hyphae occurs.

With respect to VAM fungi, direct evidence of grazing has been demonstrated only by Moore et al. (1985). In their investigation, four different microarthropods (*Folsomia candida, Onychiurus encarpatus, O. folsomi,* and *Proisotoma minuta*) were released onto agar with germinated spores of either *Gigaspora margarita, Gigaspora rosea, Glomus fasciculatum,* or *Glomus mosseae*. Feeding was observed only on *G. rosea* and *G. fasciculatum*. The hyphae were noted to have been severed rather than entirely ingested. Unfortunately, these observations were not supported by gut content analysis, which would have revealed how much of the hyphae was ingested. Although the investigation of Moore et al. (1985) demonstrates that grazing of VAM hyphae can occur, it is difficult to speculate from this evidence on whether the grazing of VAM hyphae occurs in the field and to the extent of this interaction.

Consequences of Fauna–Mycorrhiza Interactions

Types of Interaction

The soil fauna impinge on mycorrhizal fungi in three main ways: by affecting dispersal and the development of both the internal mycelium (colonisation) and the external mycelium. Of these, the effect on colonisation is a special case, since it can only be brought about by root-inhabiting animals, which in practice means pathogenic nematodes. These are known to influence the development of the internal mycelium in VAM-colonised plants (see the very thorough review by Ingham, 1988), but the mechanisms of the interaction and its significance are not yet clear. There seems to be little evidence that ECM fungi are adversely affected in this way, though some interactions certainly occur.

Effects on dispersal are better documented. VAM fungal spores have been found in the guts of a wide range of soil animals, including ants (Formicidae) and earthworms (Lumbricidae; McIlveen & Cole, 1976), woodlice (Isopoda), millipedes (Diplopoda), moth larvae (Lepidoptera) and beetles (Coleoptera: Rabatin & Stinner, 1988), and springtails (Collembola; Warnock et al., 1982; Finlay, 1985; Moore et al., 1985). It is unclear whether any of these organisms selectively feed on VAM fungal spores, but Rabatin and Stinner (1988) report that woodlouse and millipede faeces can act as inoculum to infect alfalfa (*Medicago sativa*) roots.

Some ECM fungi are certainly dispersed by animals. Fogel and Trappe (1978) demonstrated the importance of small mammals in this regard, especially for hypogeous fungi such as truffles. Epigeous fungi, in contrast, have such efficient wind-dispersal systems that it seems implausible that animals play more than a trivial role.

The ecological significance of transport by soil animals within the soil is unclear. Many VAM and ECM fungal species are widespread geographically, and it is often assumed that they are effectively ubiquitous. Sampling within defined areas, however, may reveal complex spatial patterns of inoculum density, and these might possibly arise partly through animal transport. Where fungal species interact differently with various plant species, such patterns might have important implications for community structure.

It seems likely, however, that the greatest influence exerted by the soil fauna on mycorrhizas is the consumption of the mycelium. Grazing is known to be a major factor in controlling the structure and function of the aboveground parts of ecosystems, and since it can both be observed and measured with ease, it has been intensively studied there. Grazing can, for example, cause the death of plants, reduce their competitive ability or in consequence increase that of neighboring plants, alter resource allocation patterns and especially reduce reproductive success, or even stimulate growth in isolated plants by the removal of apical dominance. Grazing on fungal mycelia can, as shown above, have a similar range of effects.

Consequences of Mycorrhizal Grazing

There are three main possible effects of grazing on a mycorrhizal fungus:

1. Grazing might limit the development of the external mycelium. This would occur if the animals were well distributed in soil or if the fungal tissue occurred in sufficiently dense patches to permit high animal population densities to persist.
2. Grazing might result in the disconnection of the external mycelium from the internal mycelium or sheath. This would occur if the animals predominantly fed in the rhizosphere, and is possible because of the unique structure of the mycorrhizal mycelium with a limited number of hyphal strands connecting the internal and external parts.
3. Grazing might stimulate fungal growth, for example, by recycling minerals locked up in senescing tissue, by importing minerals and depositing them in faeces, or by removal of growth inhibitors (see above).

The effects of such interactions on the plants are predictable. If the external mycelium is severely reduced or simply disconnected, then the internal mycelium or sheath will become parasitic, since it will still exert a demand for carbon and, presumably, other nutrients, but can offer nothing in exchange. Since this part of the mycelium may represent a significant proportion of root biomass (up to

20%), this cost may be not inconsiderable. This situation would continue until one or more of the following occurred:

1. The external mycelium might regenerate from the internal mycelium or sheath. There seems no doubt that this can occur, since root pieces can be used as inoculum, but it would certainly represent a very significant additional resource cost on the host plant.
2. Where disconnection has occurred, it is possible that some reconnection process might take place. This would involve anastomosis presumably.
3. There might be reinfection of the root system by the surviving or new external mycelium. This process requires the development of a new internal mycelium and would leave the question of the fate of the original internal mycelium unknown.

In all these cases, grazing could be expected to produce significant deleterious effects on plants, which would be specific to mycorrhizal individuals. In contrast, if grazing stimulated growth of the fungus, the predictions are more complex. It is possible that this might result in increased benefits to the host, on the grounds that if some mycelium can give a small benefit, more must give a greater one. This would imply that, as faunal population density and hence grazing pressure increased, there would be an initial benefit to the plant and then a phase of increasing disadvantage. It is equally possible, however, that an increased external mycelium, which would certainly represent a greater carbon drain on the host, would not bring any proportional benefits. This would be the case if, for instance, the growth of the plant was not limited by resources supplied by the fungus. Clearly, the net effect of grazing on the mycorrhizal association depends critically on the precise nature of the grazer–fungus interaction and the responses of the fungi to grazing. We need to examine the experimental evidence for these. Unfortunately, most of it is indirect and many critical experiments remain to be done.

Experimental Evidence

Virtually all the experimental work on mycorrhizal grazing is on VAM systems. This is odd, since ECM mycelia are very much easier to work with and their effects on plant performance are usually more dramatic. In addition, ECM mycelia represent a very large biomass in soil, are an obvious food source for the soil fungi, and are known to be eaten by soil animals (see above).

VAM fungal systems, on the other hand, have been relatively well studied, and these studies will be discussed in more detail here; it seems reasonable to suppose that similar principles will govern the interactions in both VAM and ECM mycorrhizas. Most work on VAM–faunal interactions has been in pot cultures, in which the typical, grossly simplified soil "ecosystem" of the pot experiment (sterilised soil, mycorrhizal inoculum with associated undetermined microbes, plant with different sets of undetermined microbes) is made a little more realistic by the addition of a single animal species. Such experiments have been used to demonstrate pronounced effects of collembola and nematodes, though mites, another large group of soil fungivores, have not yet been used successfully.

The typical result of such an experiment (or at least of those reported in the literature) is a reduction in the benefit to the plant of being mycorrhizal. This was the case when Salawu and Estey (1979) added the nematode *Aphelenchus avenae* to soybean cultures inoculated with a *Glomus* sp. In the absence of nematodes, mycorrhizal plants grew better than nonmycorrhizal ones, but when nematodes were present, shoot yield was reduced by 40% and nodulation by nearly 90%. Similarly, Warnock et al. (1982) found that the collembola *Folsomia candida* reduced the yield of mycorrhizal leek plants (infected with *Glomus* strain E3) to levels similar to those of nonmycorrhizal plants (Fig. 10.1). P concentrations were actually lower in mycorrhizal plants with collembola than in nonmycorrhizal plants, while P inflows declined from 68 fmol/cm/sec in mycorrhizal plants without collembola to 17 when collembola were added. These figures compared to values of 23 and 10 for nonmycorrhizal plants; none of the three lower values was significantly different from each other.

These differences were not the result of different intensities of infection in the roots, since plants were inoculated 8 weeks before collembola were introduced, and between 74 and 84% of the root length was infected in all cases after 12 weeks. Nor could direct effects of collembola on the plants, such as root grazing, be implicated, since nonmycorrhizal plants were unaffected and root weight ratios unaltered. The guts of the collembola contained fungal material, but nothing recognisably of root origin, and the population density of collembola in mycorrhizal pots was three times that in nonmycorrhizal pots after 12 weeks. The two main treatments were combined factorially with a third: the addition of soil leachings, to restore soil bacteria and other fungi. This increased plant growth (by between 25 and 90%, depending on treatment) but had no effect on P inflows or collembola population growth.

Taken together, all this evidence strongly points to the likelihood that collembola and nematodes can influence plants by damaging the external mycelium of VAM fungi, so reducing P inflows and the benefit to the plant of infection. In some experiments, however, there may be no effect of adding animals: Hussey and Roncadori (1981) allowed the nematode *Aphelenchus avenae*, which Salawu and Estey (1979) had found seriously to inhibit VAM function in a soybean–*Glomus* association, to feed on cotton infected with *Gigaspora margarita* and *Glomus etunicatus*. They could only find inhibitory effects at unrealistically high nematode densities, which could not in any case be sustained.

Some of the most detailed studies are those of Finlay (1985), who performed a series of experiments in which he varied the species of plant, fungus, and collembola, and also investigated the role of collembola density. He was able to

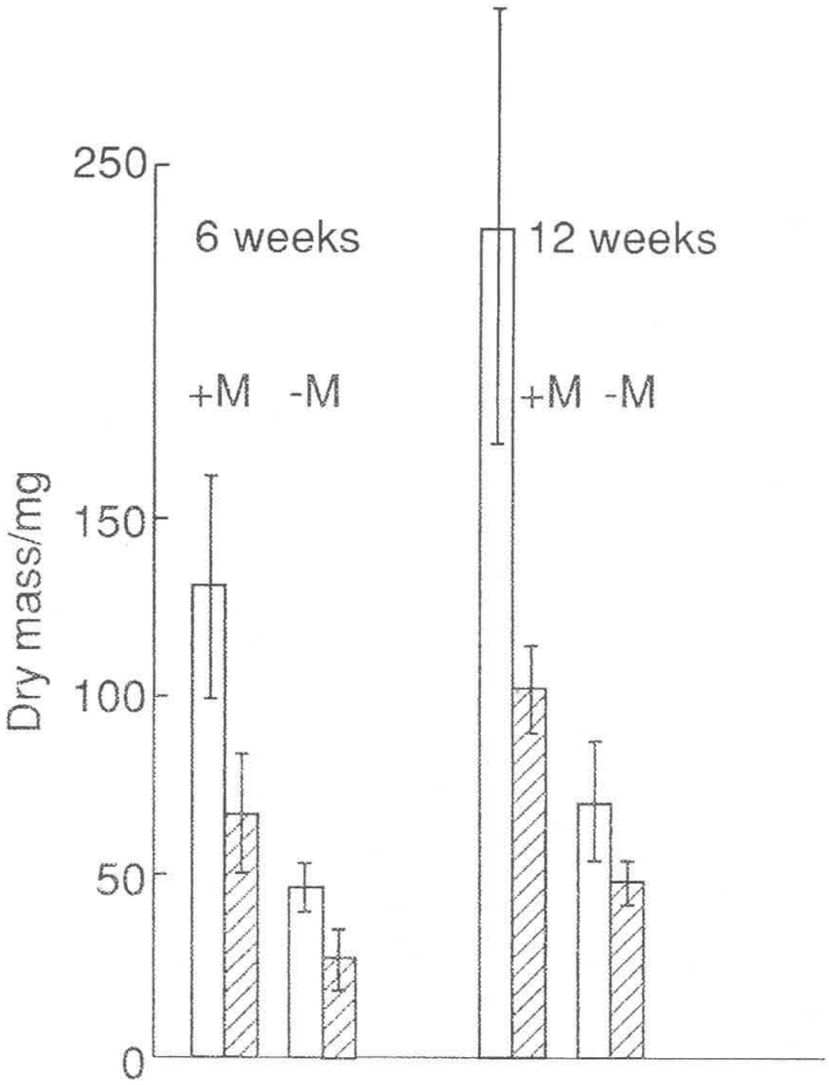

Figure 10.1. Influence of the collembola *Folsomia candida* on dry mass production in leek *Allium porrum*, infected (+M) or not (−M) with *Glomus* strain E3. Hatched columns represent pots containing *F. candida:* bars represent 95% confidence limits. Data from Warnock et al. (1982).

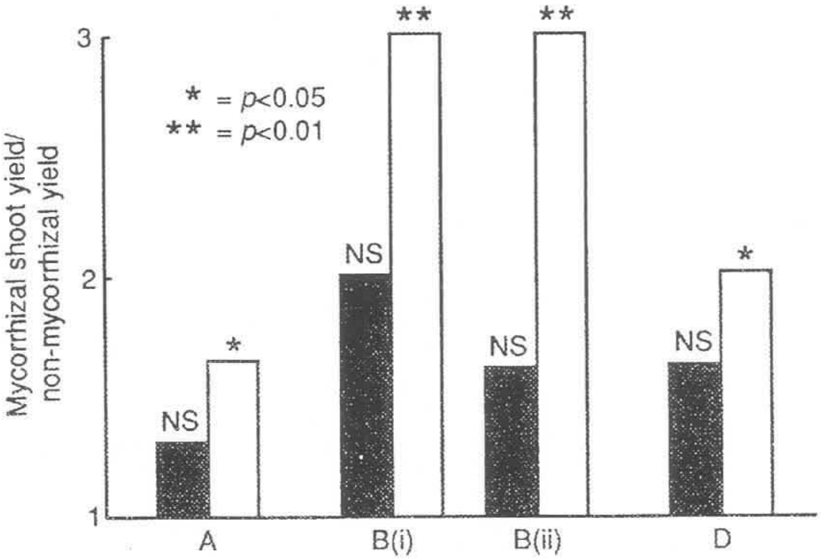

Figure 10.2. Mycorrhizal stimulation of shoot yield in a range of laboratory experiments (A–D) with (▩) and without (☐) collembola added to pots. Significance levels refer to *t* tests between the means of ln-transformed mycorrhizal and nonmycorrhizal treatments; the data are presented here as the quotient of yield in mycorrhizal pots to that in nonmycorrhizal pots. The experimental details were as follows (plant, fungus, collembola): (A) *Allium porrum, Glomus tenue, Onychiurus ambulans;* [B(i)] *A. porrum, G. fasciculatum, Folsomia candida;* [B(ii)] *A. porrum, G. fasciculatum, O. ambulans;* (D) *Trifolium pratense, G. caledonium, O. ambulans.* From Finlay (1985).

confirm the stimulation of collembola growth by mycorrhizal infection, and found that increased plant size brought about by phosphorus fertilisation was ineffective in promoting collembola numbers, whereas infection by a mycorrhizal strain (*Glomus tenue*) that did not promote plant growth, did stimulate animal numbers. When considered with the demonstration that *Onychiurus ambulans* guts contained fungal spores and hyphae, these results confirm that the effect of collembola on the association is initially on the fungus not the root.

In four of five experiments, Finlay (1985) showed that mycorrhizal infection stimulated plant growth only in the absence of collembola (Fig. 10.2). Since these experiments used two species of plant, two of collembola and six of *Glomus* (not all combinations are shown in Fig. 10.2), their generality seems good. Most striking, however, was the nonlinearity in the relationship between plant P uptake and collembola density (Fig. 10.3): low densities of collembola increased P uptake, whereas high densities decreased it. This is what would be expected if either mycorrhizal biomass was supraoptimal (from the plant's standpoint), so that grazing initially brought it to an optimum level and then reduced it below

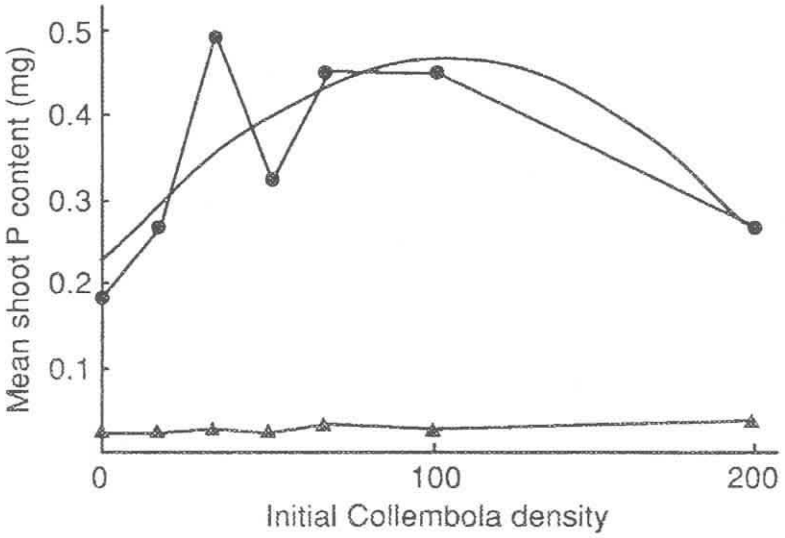

Figure 10.3. Relationship between mean shoot P content and initial collembola density for mycorrhizal (●) and nonmycorrhizal (▲) *Allium porrum* plants grown with seven densities of *Onychiurus ambulans* and with *Glomus fasciculatus* as the mycorrhizal symbiont. Data are from a pot experiment (Finlay, 1985). The line shown for mycorrhizal plants is a fitted quadratic relationship.

that, or grazing stimulated fungal growth and initially raised it from a suboptimal level. The former seems, however, to be more likely to give the observed effects (see Fig. 10.4).

Recently, Harris and Boerner (1990) completed experiments very similar to those of Warnock et al. (1982) and Finlay (1985), except that the plant was the woodland herb (and occasional weed) *Geranium robertianum*. They too found that collembola reduced VAM infection and that the effects of initial collembola density on plant growth were nonlinear. There also was some evidence for parallel changes in P uptake. Unfortunately they did not measure collembola densities at the end of their experiments and so it is not possible to conclude that the collembola were actively utilizing fungal resources.

In pot experiments, therefore, it has been demonstrated that soil animals, especially collembola and nematodes, feed on the external mycelium of VAM fungi and in so doing reduce the P inflow to plants relative to animal-free conditions. Plants do not normally grow in the absence of soil animals, so it is reasonable to suppose that these effects will be of particular importance under field conditions. Unfortunately, a conclusive demonstration of the effect in the field is technically difficult.

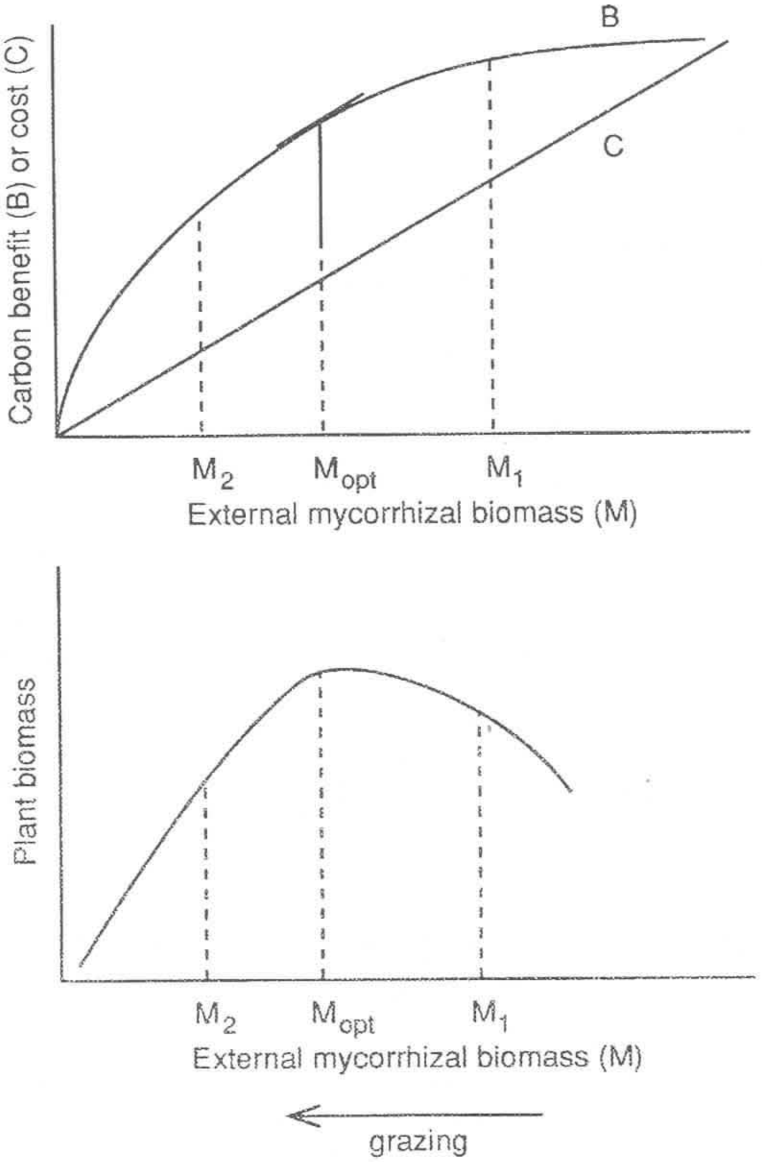
B
C
Carbon benefit (B) or cost (C)
M$_2$
M$_{opt}$
M$_1$
External mycorrhizal biomass (M)
Plant biomass
M$_2$
M$_{opt}$
M$_1$
External mycorrhizal biomass (M)
grazing

Figure 10.4. Diagrams to illustrate cost–benefit analysis of mycorrhizal grazing. (a) Carbon cost (C) is assumed to be a linear function of external mycorrhizal biomass. Carbon benefit (B) is the additional carbon fixed because of increased P uptake. The optimum mycorrhizal biomass is found where $dB/dM = dC/dM$. (b) Grazing reduces external mycorrhizal biomass from its initial value (M_1) through M_{opt} to a lower value (M_2), with consequent effects on plants growth.

Field Experiments

Pot experiments that include soil animals represent a significant increase in realism over the traditional mycorrhizal pot experiment. One development from these would be the construction of microcosms containing gnotobiotic systems in which to study mycorrhizal responses. This has not yet been done, though workable microcosm systems have been used to investigate decomposition and nutrient cycling. An alternative approach is to move directly into the field and to measure mycorrhiza–fauna interactions directly, either by observation or manipulation.

The most obvious manipulation to make is the application of a biocide to reduce or eliminate the populations of fungivores. This is a very crude manipulation, since no biocides are specific for fungivores, and indeed nearly all those used to date have very broad-spectrum activity. One of the first such studies was that of Finlay (1985), who applied the insecticide "chlorfenvinphos" (diethyl 1-(2′, *d*′-dichlorophenyl)-2-chlorovinyl phosphate) and the fungicide "benomyl" to soil in plots in a field that had been fumigated with methyl bromide 10 months previously. All plots received an inoculum of 2 kg/m^2 of *Glomus occultum* inoculum, and were sown with red clover *Trifolium pratense*. Benomyl, which is an effective anti-VAM agent (Hale & Sanders, 1982; Fitter & Nichols, 1988), reduced shoot mass, P contents, and P uptake rates (expressed as rate of P accumulation per unit shoot mass).

The insecticide treatment reduced collembola numbers by 80%; other soil animals were not examined. Plants in insecticide treated plots had higher shoot mass, shoot P concentration, shoot P content, and shoot P accumulation rates (Fig. 10.5). The increases were not due to changes in P availability, and the results are consistent with the notion that reducing collembola numbers permitted better mycorrhizal function. The increase in shoot mass following insecticide treatment could be due to reductions in root-feeding insects, but the effect on P accumulation rate is not so obviously explained in this way.

This study was performed in a relatively P-deficient soil under agricultural conditions likely to maximize the potential contribution of mycorrhizal fungi to plant growth. In natural and seminatural communities the evidence for VAM benefits to plants is much less convincing (Fitter, 1989), and so the chances of obtaining positive responses to the reduction of grazing pressure are less good. Nevertheless, one of the most obvious possible reasons for the failure of VAM to produce good responses in such communities is grazing (Fitter, 1985), so it is important to extend these studies. McGonigle and Fitter (1988) applied insecticide, again chlorfenvinphos as in Finlay's (1985) study, to a seminatural, species-rich grassland at Wheldrake Ings, Yorkshire (England). They monitored the phosphate nutrition of the grass *Holcus lanatus*, an abundant species in the community. As previously, insecticide reduced collembola density 3-fold and significantly increased shoot mass, especially at the end of the experiment, 69

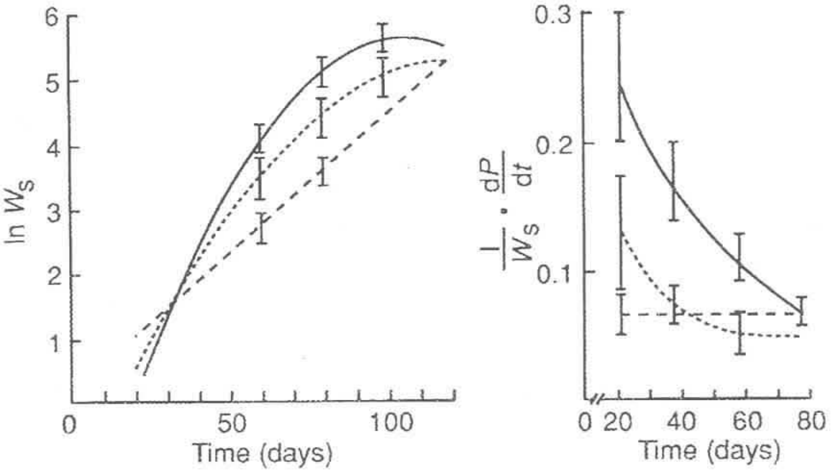

Figure 10.5. Changes in (a) mean shoot mass m^{-2} and (b) rate of phosphorus accumulation into shoots in *Trifolium pratense* plants grown in field plots treated with insecticide (– – –), fungicide (——), or untreated (···). Bars represent 95% confidence intervals to fitted curves. From Finlay (1985).

days after first application. At this time there was also a large increase in P flux, from 70 fmol/cm^2/sec in the controls to 230 in the insecticide-treated plots. P fluxes (uptake rate per unit root surface area) were calculated rather than inflows (uptake rate per unit root length) because the very fine roots of *H. lanatus* gave very low inflow values.

This experiment provides evidence similar to that of Finlay (1985), but for a seminatural community. Various explanations are possible, but the increased P uptake rate per unit root length or area is hard to explain by any mechanism other than one involving mycorrhizas. The increased P fluxes in *H. lanatus* coincided with the onset of flowering, which is likely to increase P demand by the plant considerably. It is possible that plants growing in the field under natural conditions normally have low P demands that can be satisfied by diffusive flux through soil, thus rendering the mycorrhizal association superfluous, but that at certain times in the life-cycle, of which sexual reproduction is an obvious example, P demand is much greater, rendering the mycorrhizal flux highly beneficial. This interpretation is supported by the data of Dunne and Fitter (1989) for cultivated strawberry: they measured P inflows of 13 fmol/cm/sec in the vegetative phase rising to 435 fmol/cm/sec at fruiting. Such high values could not possibly be achieved by diffusive flux. If converted to a flux across the root, the highest inflow for strawberry represents 6.9 pmol/cm^2/sec, higher than reported for any other mycorrhizal plant (McGonigle & Fitter, 1988) and higher than the maximum P fluxes reported for solution culture experiments (Barber, 1984; p.216).

These data are consistent with the hypothesis that soil animals do consume significant amounts of VAM fungal hyphae under realistic field conditions and so render the association less effective. Since VAM often give little obvious benefit to plants in the field, possibly because of inherently low P demand, it is not surprising that another attempt to manipulate a natural ecosystem using biocides, that by Ingham et al. (1986), produced few mycorrhizal responses. In this study various biocides were added to undisturbed prairie vegetation; carbofuran, a nematicide, increased VAM infection in roots of *Bouteloua gracilis*, but without altering shoot P concentrations. This agrees with the study by Fitter (1986) in an alpine grassland, in which reducing VAM infection in the roots of various species actually increased shoot P concentration. In neither case were plants receiving an obvious quantitative benefit from infection.

Other experiments currently in progress may give further insights into this complex system. For example, Brown and Gange (1989), studying the role of herbivory in succession, found very large changes in plant species composition following the application of insecticide to soil. Whether these might be related to effects on mycorrhizal grazers remains to be seen. What is urgently needed is a study in which the interaction between the soil fauna and mycorrhizal fungi is studied throughout the life-cycle of a plant species. Until this is achieved, it will remain difficult to offer more than conjecture in this area, although there seems little doubt that mycorrhizal fungi are an important food resource for soil fungivores, and that these animals can radically alter the functioning of the symbiosis. Any attempt to understand the role of mycorrhizas in either natural or agricultural systems must take grazing into account.

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